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# The Effects of the Far-Infrared Ray (FIR) Energy Radiation on Living Body

Kikuji Yamashita

Additional information is available at the end of the chapter

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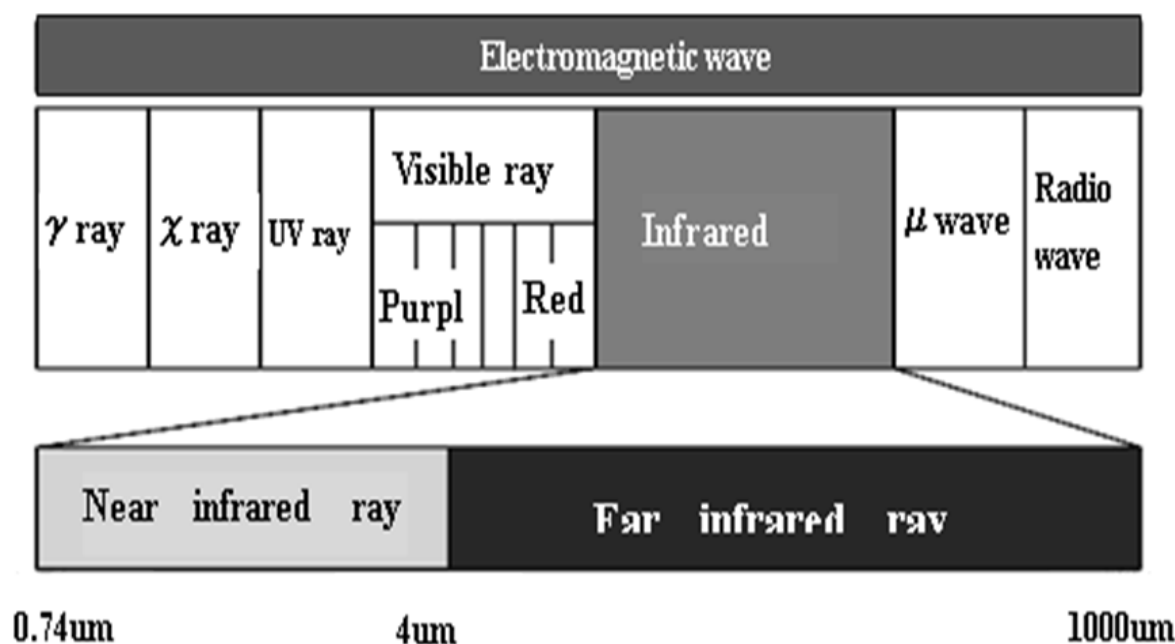
## 1. Introduction

### 1.1. A far infrared ray (FIR) energy

The power of the energy radiated from any material is dependent on the temperature. For example, the sun with the temperature of 6,000°C at the surface and ~15,000,000°C at the depths radiates the radio-magnetic ray with from the weak to the strong energy. The radio-magnetic ray with strong energy is absorbed before the arrives at the surface of the earth. But, if a human bathe in the ultraviolet ray even if it arrives only about 0.6% of whole ultraviolet ray radiated by the sun, the surface of skin is burned and strongly damaged. If the cell is poured the large quantities of ultraviolet ray, the DNA of nuclear is injured to induce a liver spot, freckles and aging of skin. Still more, it is thought that the immunity is inhibited and a cataract and a skin cancer can be induced by the ultraviolet ray. It was thought that the sunlight arrived at the surface of earth was finally composed of the ultraviolet ray (UVA, UVB) of 6%, the visible ray of 52% and the infrared ray of 42%. It was well known that the energy of these lights bring a lots of the good effects for living bodies. The other sides, the materials on the earth we are living radiated the narrow range of energy from 5 to 50μm in FIR under 30°C by getting the energy of sunlight.

In other words, thought the radiating energy on the earth creating a life was only FIR of 5~50μm by getting the energy of visual ray and FIR, the earth was filled with the energy of only FIR of narrow range. It was means that the energy of FIR may make contribution to the birth of a life. The National Aeronautics and Space Administration (NASA) reported which ray indispensable for the maintenance of the life was FIR of 4~20μm as the artificial sun in the space station. Therefore, it comes to a conclusion that the FIR is the raising ray for the living body (Fig.1). Recently, there have been many studies of the effects of FIR on health and food preservation. The available evidence indicates that whole-body FIR irradiation has

biological effects [1-7]. Whole-body FIR irradiation is believed to improve human health and sleep by enhancing blood circulation in the skin [1, 2]. However, the effects of FIR on cells are not clearly understood. These thinking let me realize that there are a lot of natural materials radiating strongly the energy of FIR as a charcoal, stone, soil and tree. So, these natural materials were being used for our studies on the energy of FIR.



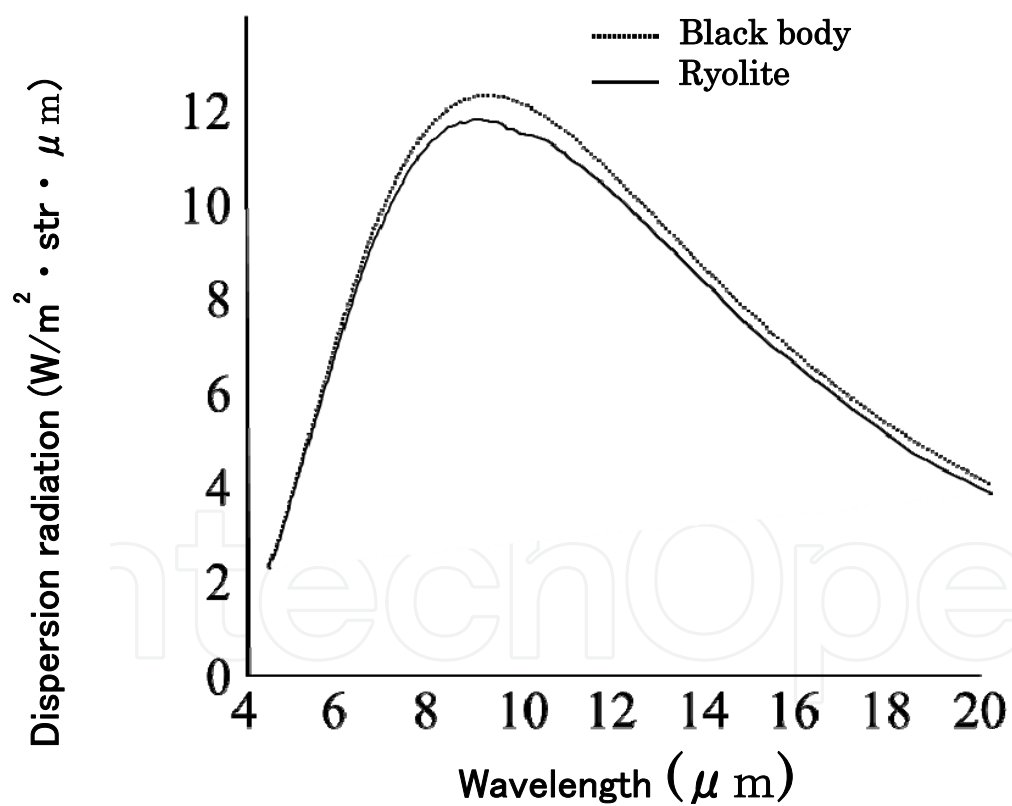
**Figure 1.** The classes of the electromagnetic wave by the wave length  $\mu$ m

## 2. The characteristics of rhyolite

In order to study strictly the effects of the energy of FIR in 4~20 $\mu$ m on the living body, the radiating machine of the energy of FIR should be developed. In order to develop it, the selection of a good FIR radiator were started at first. The most noticeable effect of FIR energy radiation was the activation of water molecules. The change of the weight of the ultrapure water on the FIR ceramics containing the five natural stones, 4 chemical products of mineral oxide and charcoal was exactly measured with time at 37°C. Though it was made clear that some natural stones activated the evaporation of pure water, the rhyolite especially strongly activated. Then, the rhyolite (MATERA Inc. Toon, Ehime, Japan) mined at Toon, Ehime in Shikoku Island was selected as the FIR radiation ceramics for the development of the radiating machine of the energy of FIR (Fig.2). The characteristic of rhyolite was the volcanic rock containing 70% over silicate dioxide with the flowing patterns formed by the phenocryst of magma (Fig.2). Though the components are the quartz, feldspar and biotite, the rhyolite is similar the granite. When the FIR energy radiated from the rhyolite was measured by Fourier transformed infrared spectrophotometer, it was made clear that the rhyolite was radiated 90% over the ideal black body at whole range of 5~20 $\mu$ m (Fig.3). It was proved that the rhyolite was the excellent radiator of FIR energy of 5~20 $\mu$ m.



**Figure 2.** The photograph of the rhyolite



**Figure 3.** The radiation energy of the far infrared ray from the radiator

The powder of rhyolite showed the antibacterial action to the *Staphylococcus aureus* ATCC6538P at 1/5012. The adhesive sheet with the 15% powder of rhyolite showed at 1/1995. Still more, the paint containing the 10 % powder of rhyolite showed the antibacterial action to the Methicillin-Resistant *Staphylococcus Aureus* MRSAIID 1677 at 1/5012.

The values of antibacterial action of the materials containing rhyolite are respectively 3.7, 3.3 and 3.2. Though the values of antibacterial action over 2.0 was estimated as the antibacterial substance, the rhyolite was regarded to be the fairly mighty the antibacterial substance. Still more, the antibacterial action of rhyolite to the colon bacillus was verified in neutral condition at our laboratory also (Fig.4, Table 1). These results suggested that the antibacterial action of rhyolite did not depend on the change of pH. This graph showed the effects of rhyolite on the oxidation-reduction potential of water (Fig.5).

Item	The water treated with the powder of rhyolite	The water treated with the stone of rhyolite	Control
Biochemical oxygen demand mg/1	2.1	2.4	2.4
Chemical Oxygen Demand mg/1	3.4	3.9	3.9
Suspended solids	12	10	8
Dissolved Oxygen	9	8.9	9.1
Escherichia coli group number MPN/100ml	0	2200	14000
Conductivity	18.6	19.5	19

Table 1. The chemical analysis of the rhyolite treated water

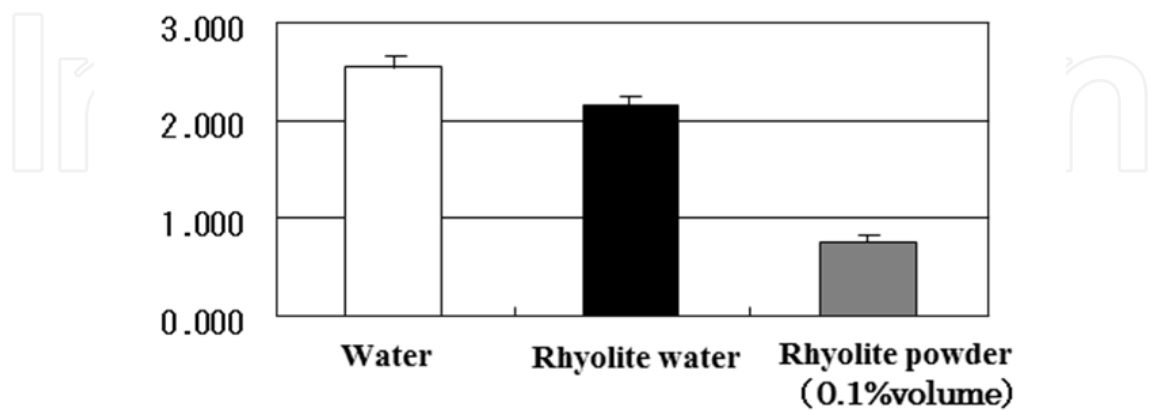
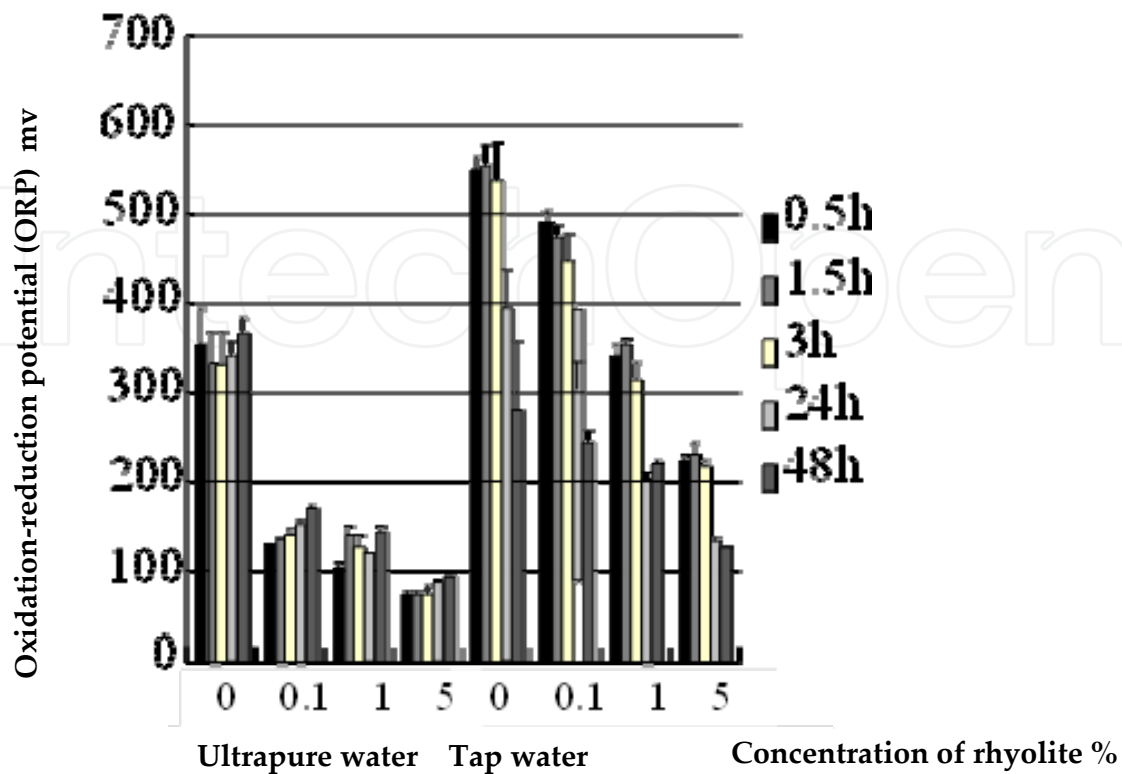


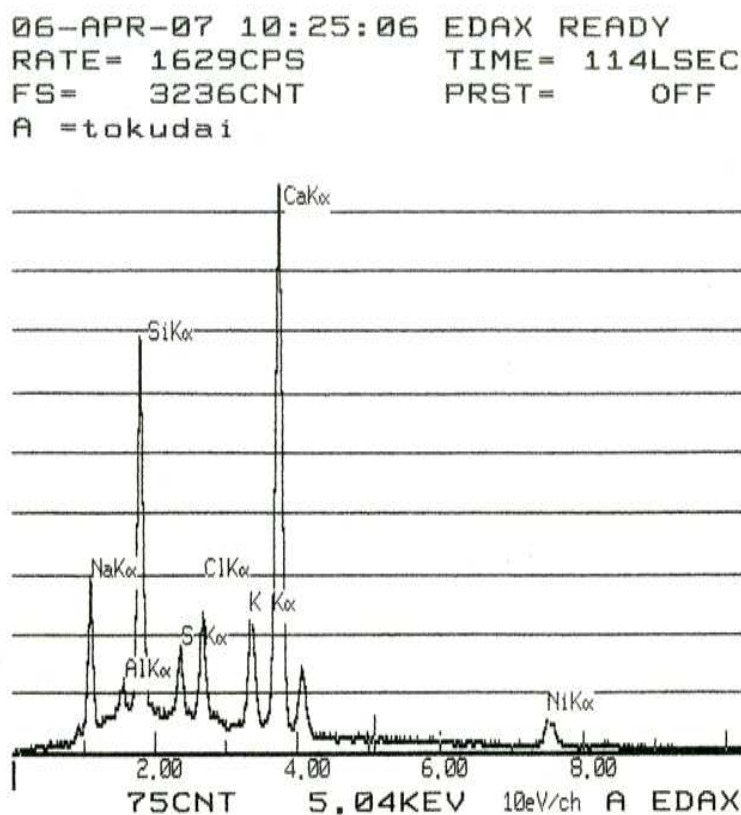
Figure 4. The effect of the rhyolite on Escherichia coli; Escherichia coli (DH50) were cultured for 16 hours in the sterilized pure water, the water containing the rhyolite and the water containing 0.1% rhyolite powder. The proliferation of the Escherichia coli inhibited at 15.6% in the water containing the rhyolite and 69.9% in the water containing 0.1% rhyolite powder.



**Figure 5.** The change of the oxidation-reduction potential affected by the rhyolite. The oxidation-reduction potential (The longitudinal axis shows ORP, mv) decreased depending on the contents of the rhyolite(The transverse axis shows %) after 0.5-48h.

Though the oxidation-reduction potential of tap water is about 550, it decreased with time to be about 280 after 48 hours. When the powder of rhyolite was put into tap water at 0.1%, 1%, 5%, the oxidation-reduction potential of tap water decreased respectively up to 480, 340 and 220. Then, it decreased with time up to about 130 after 24 hours. It was thought that the high oxidation-reduction potential of the tap water caused by the residual chlorine. The reason why the oxidation-reduction potential of the tap decreased with time might be that the hypochlorous acid in tap water evaporated as the gas of chlorine with time. The oxidation-reduction potential decreased in the dependent upon the density of the rhyolite powder. It was thought that the reason was by the elution of any reducing agent from rhyolite. The small amounts of any elements with oxidative reaction were left in the ultrapure water. Therefore, the small amounts of rhyolite powder could reduce the most of oxidizers in the ultrapure water. The mighty oxidative powers of the residual chlorine attack the cell membrane of a microorganism and a virus to degenerate the inner protein and show the germicidal and disinfectant effects. It was thought that the reducer generally do not show a mighty antibacterial effect. Why the rhyolite shows the mighty antibacterial effects? Then, the 500g of rhyolite powder was put into 1l of ultrapure water. After keeping 18hours, the supernatant of the water was collected and filtrated with the filtering paper. Still more, the filtrate was centrifuged at

3000rpm for 10min. The supernatant of 600ml dried at 200°C for 5 hours up to get 13.4mg of white precipitates. The precipitates were analyzed by the x ray diffraction and the electron-micro analyzer. The results showed that the precipitates mainly consist of calcium and silicon (Fig.6). As the rhyolite contained 70% over silicate, the results suggested that the ceramics structure consist of silicate oxide should disintegrate. Then, the silicate radical molecule containing an additional electron could be formed. It was thought that the radical molecules are so active that the bacterium *el.al* was contacted to collapse the cell membrane with an antibacterial action. Though the mechanism to form the silicate radical molecule was not clear, the antibacterial effects of the rhyolite containing the 70% over silica were the experimental facts.



**Figure 6.** The analysis by the x ray diffraction of the components in the solution

### 3. The development of the FIR energy radiator

In order to clarify the effects of the FIR energy radiation on the living body at the rebel of a cell and animal, the FIR incubator for cell culture and animal raising apparatus was developed utilizing the valid ceramics as the rhyolite and the pureblack that the resemble effects as the ideal black body are well known.

The CO<sub>2</sub> incubator must be exactly controlled the temperature inside of the incubator at 37±0.5°C. Therefore, though the water jacket of about 40litter is equipped for the suppression



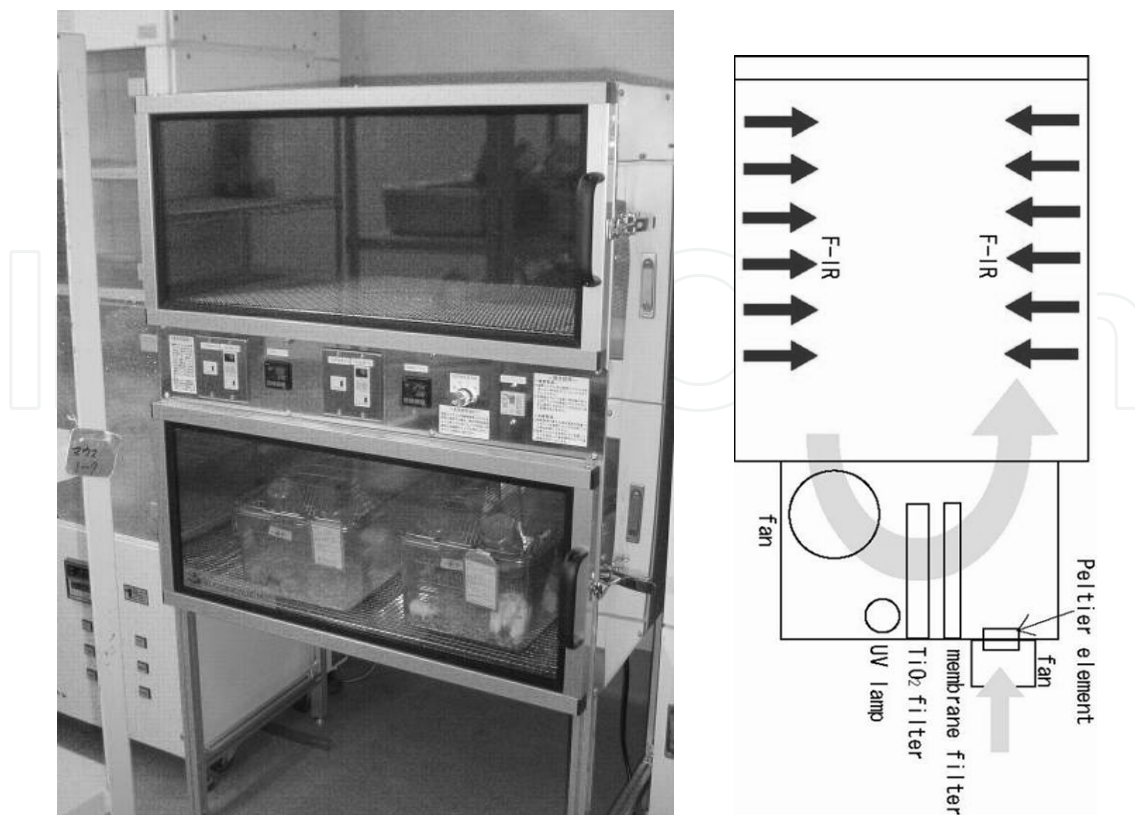
of heat loss, the equipment of incubator becomes solid and heavy. Then, 0.5% CO<sub>2</sub> gas need be supply into the incubator to activate the culture cell breathing. Still more, the gas of 0.5% CO<sub>2</sub> in air must be supplied though the water layer in order to keep the humidity near 100%. So, we remodel the existing CO<sub>2</sub> incubator. The heating system was changed to FIR panel heater for keeping the temperature in the incubator at  $37\pm0.5^{\circ}\text{C}$ . The five face at up, down, both side and behind in the incubator except the front glass were coated with the valid ceramics as the rhyolite and the pureblack close to black body for raising the efficiency of FIR radiation. The all shelves were also coated with the same ceramics. Still more, the water jacket was change to the simple insulation, because the FIR energy directly and instantly heats the surface of objects even if the air and space lie between the surface of incubator and the objects. As a result, the light and compact CO<sub>2</sub> incubator that can continuously radiate the FIR energy for 24hours was developed with a great success (Fig.7) [8]. So, the strict comparative experiments on the effect of the FIR energy radiation on the culture cells become possible utilizing the same type of CO<sub>2</sub> incubator without remodeling as control.



**Figure 7.** The FIR CO<sub>2</sub> incubator

Then, the FIR animal raising apparatus with same FIR radiating system of the FIR CO<sub>2</sub> incubator to control the temperature inside  $20-40^{\circ}\text{C}$  at will and break down any smell chemical products by photo-degradation with the light catalyst of TiO<sub>2</sub> could be also developed (Fig.8). The FIR animal raising apparatus has the two chambers which the upper chamber is coated with the valid ceramics as the rhyolite and the lower chamber is no coating [9]. So, the strict comparative experiments on the effect of FIR energy radiation on animals become also possible by using the FIR animal raising apparatus.





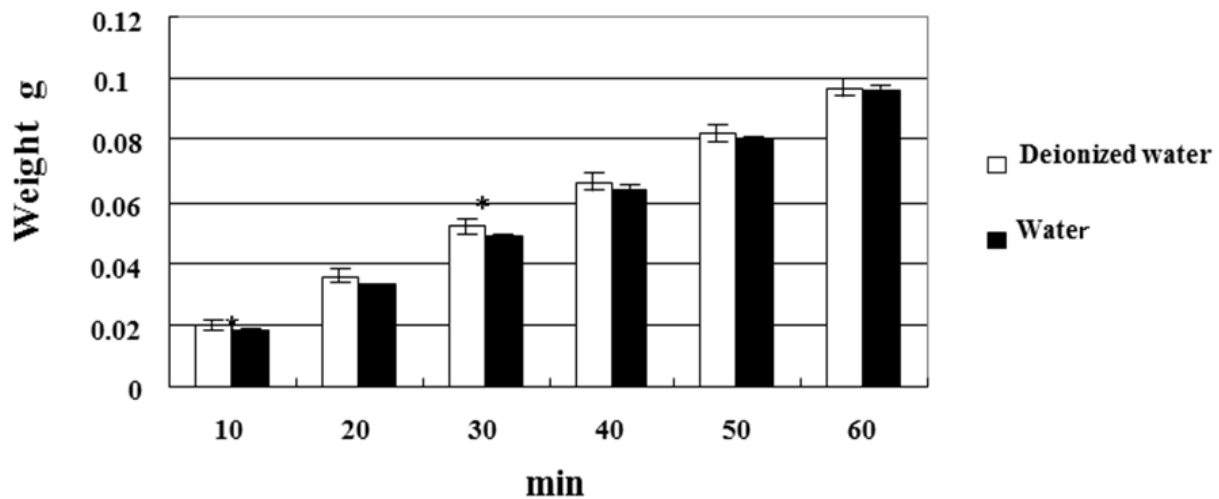
**Figure 8.** The FIR animal raising apparatus

## 4. The effects of FIR energy radiation on water

### 4.1. The volatility and fluidity of water

Water is indispensable for human life. As water is generally present surrounding us, the characteristics of water is hard to be considered as a strange matter. But, the truth that a boiling point of water is  $100^{\circ}\text{C}$  is peculiar comparing with the other elements with the similar molecular weight. For examples, though a sulfur is next of oxygen in the periodic table of the elements, the boiling point of hydrogen sulfate ( $\text{H}_2\text{S}$ ) is  $-60.7^{\circ}\text{C}$ . But, as the molecular weight of oxygen is smaller than that of sulfur, the boiling point must be smaller than  $-60.7^{\circ}\text{C}$ . It is supposed that the boiling point of water should be theoretically at about  $-80^{\circ}\text{C}$ . Then, the coagulating point of water is appropriate theoretically at about  $-110^{\circ}\text{C}$ . Well, why the boiling and coagulating points are respectively at  $100^{\circ}\text{C}$  and  $0^{\circ}\text{C}$ ? The reason is by the formation of cluster through the pulling against each other among the water molecules by a hydrogen bond et al. The imaginary molecular weight inferred from the boiling and coagulating points is about 100 by which 5, 6 water molecules gathered. Then, a water molecule has as much as four hydrogen bonds. Moreover, it was thought that the shapes of the cluster shows a straight chain, from a square to eleven-cornered shape, and the average shape is the pentagon. Moreover, it was thought that the cluster of water is not stable for long time, but it was formed and broken at the cycles of  $10^{-12}$  seconds. But, this condition of cluster is only applied to the very pure water without any

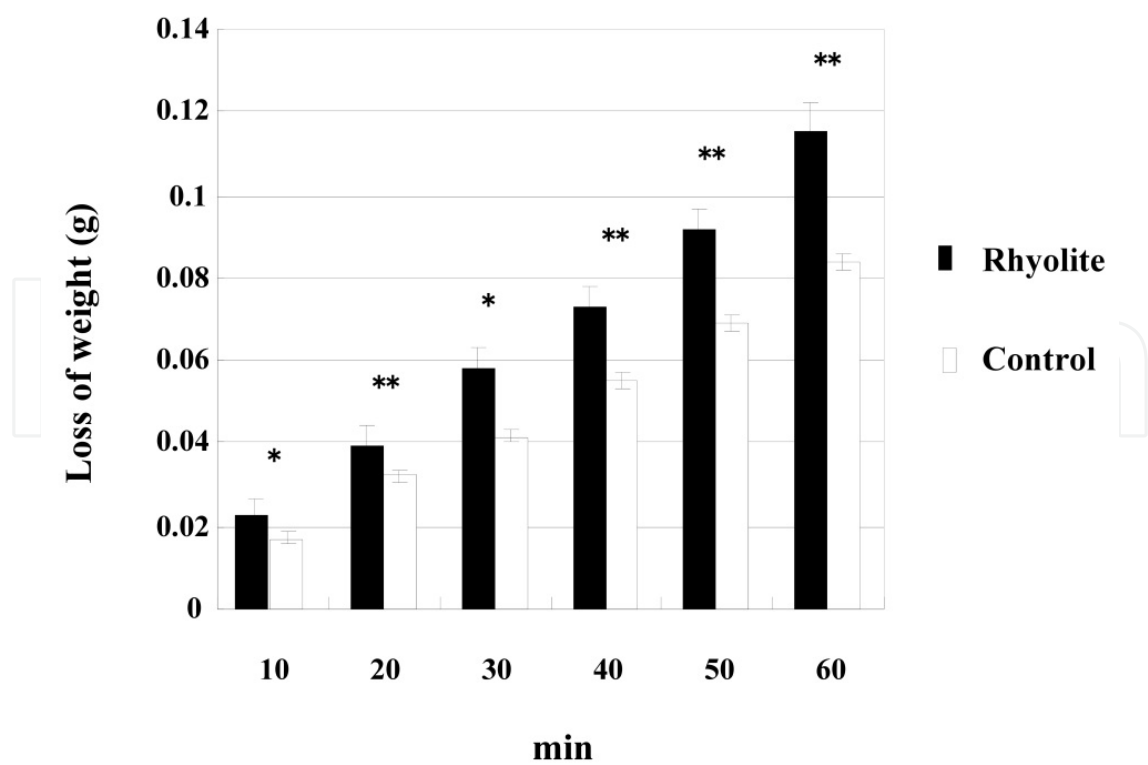
ion and impurity. The real water actually contained various impurities. Even if the very pure water is kept in air, the condition of water changes moment by moment by melting of a gas and molecules from air. Then, in order to estimate the present conditions of energy in the water, the change of the weight of water is exactly measured for the indicator of the volatility. When the difference of volatility between the tap water and the deionized water are compared, the deionized water is clearly volatilized more quickly than the tap water (Fig.9).



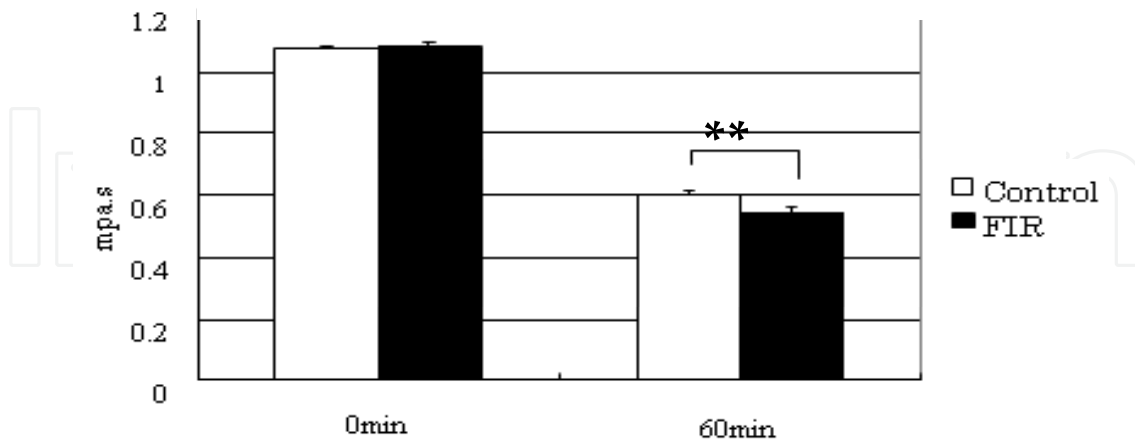
**Figure 9.** The change of the weight of the deionized water and the tap water

The high volatility means that a lot of water molecules spring out of the surface of water. Therefore, it is supposed that the energy of the water molecules is high and its cluster is small. This graph shows the change of weight of the deionized water radiated FIR energy through the glass layer by keeping in the FIR incubator for 12 hours and the untreated deionized water (Fig.10). Though the deionized water more quickly losses the weight than the tap water, the deionized water radiated FIR energy losses much bigger weight than the deionized water. In other words, it was made clear that the deionized water radiated FIR energy has high energy in the molecules to evaporate much easy. The results mean that the cluster of the water can become small by the FIR energy radiation. If that is the case, why the cluster formed by the water molecules changes by accepted the FIR energy? The water molecule consisting of two [O-H] bonds opening with  $104^\circ$  has three types of the vibration energies. One is the deformation vibration of  $1594\text{cm}^{-1}$  and the others are the symmetrical and asymmetrical expansion and contraction energy of each  $3656\text{ cm}^{-1}$  and  $3756\text{ cm}^{-1}$ . When the energy were convert to the wave length, the deformation vibration is  $6.27\mu\text{m}$  and the expansion and contraction energy are  $2.74\mu\text{m}$  and  $2.66\mu\text{m}$  [10]. If the FIR energy of  $5\sim 20\mu\text{m}$  radiated the water molecules, the deformation vibration of the water molecule was activated by the transmission of the energy through the resonance effect because the energy of deformation vibration was coincident with the radiating energy. Consequently, it was thought that the kinetic energy

of the water molecule increased to rush out of a cluster. The cluster in the activated water by FIR energy radiation ought to raise the volatility. On the contrary, the strength of FIR energy radiation by any materials can be estimated by measuring the change of the volatility. Then, when the boiling points of water was measured, that of FIR radiated water was 97.4°C and that of control water was 98.9°C. The boiling point of water decreased clearly by FIR energy radiation. These results also suggested that the water molecules are at the condition to rush out easy from the surface of water. Still more, when the viscosity of water was measured, it was clarified that the viscosity clearly decreased by the FIR energy radiation (Fig.11). These results also suggest that the water become to flow easy by the same change of the cluster in water. It is thought that the water radiated FIR energy increases the volatility and fluidity at the same time. It is a very important finding. For example, it is thought that the blood and lymph flow in the human body become smoothly and activates by radiation of the FIR energy. It is only natural that the collection of lymph fluid into a lymph vessel rides the swelling in the body. Then, a tear, sweat and digestive juices become similarly to flow easy also. It is suggested that the activation of the secretion of a tear stimulate the para-sympathetic nerve system. Still more, the activation of the secretion of a sweat stimulates the various metabolisms in whole body. The activation of the secretion of the saliva and digestive juices stimulates the digestive function to make the body vigorously.



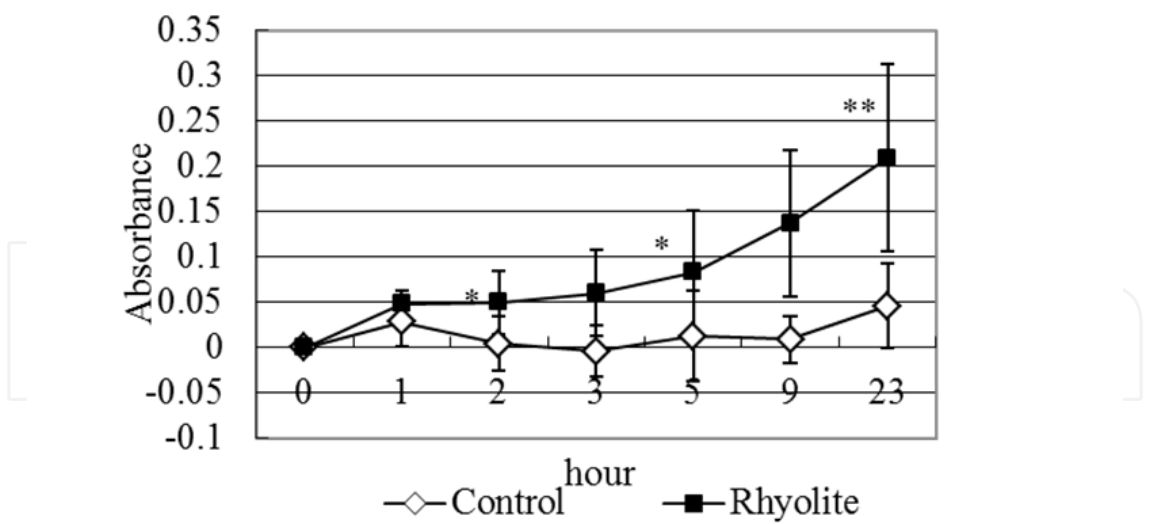
**Figure 10.** The change of the deionized water weight affected by the rhyolite as the FIR radiator



**Figure 11.** The change of the viscosity of water by the rhyolite as the FIR radiator

#### 4.2. The solubility of water

It was found that the water radiated FIR energy by the rhyolite become to dissolve variable products. The plastic culture dish adhered proteins as bovine serum albumin (BSA) were prepared for the estimation of the solubility of water. The FIR energy radiated ultrapure water by the rhyolite and non-treated same water poured into the dish, and was collected for measurement of the UV absorbance for protein. The results showed that the FIR energy radiated ultrapure water could dissolve much more BSA than the non-treated same water (Fig.12). Not only protein but also sugar and sodium hydroxide were checked the solubility. The results showed that the FIR energy radiated ultrapure water could dissolve much more them. When the rhyolite powder was inserted into a fish tank at about 0.5%, the water in the tank became clear just after 4 days. These results were considered by which the deposited protein and other products in the tank dissolved in water to raise the degree of transparency by the FIR energy radiation from the rhyolite. It was confirmed that the fishes and shell fishes become live longer than usual and keep the freshness in the tank with the rhyolite. For the application of these effects of the FIR energy radiation on water, the development of the contact lens cleaner was tried. The force of cleaning in the FIR energy radiated ultrapure water were estimated to the contact lens attached the BSA on the surface. The results suggested that the force of cleaning of water was surely raised by FIR energy radiation from rhyolite. But, as the force of FIR was defeated to a detergent, the development of the contact lens cleaner was given up. However, it was expected that the effects of the FIR energy radiation from rhyolite, on which the force of solubilizing in water was activated, will be applied in various fields in future.



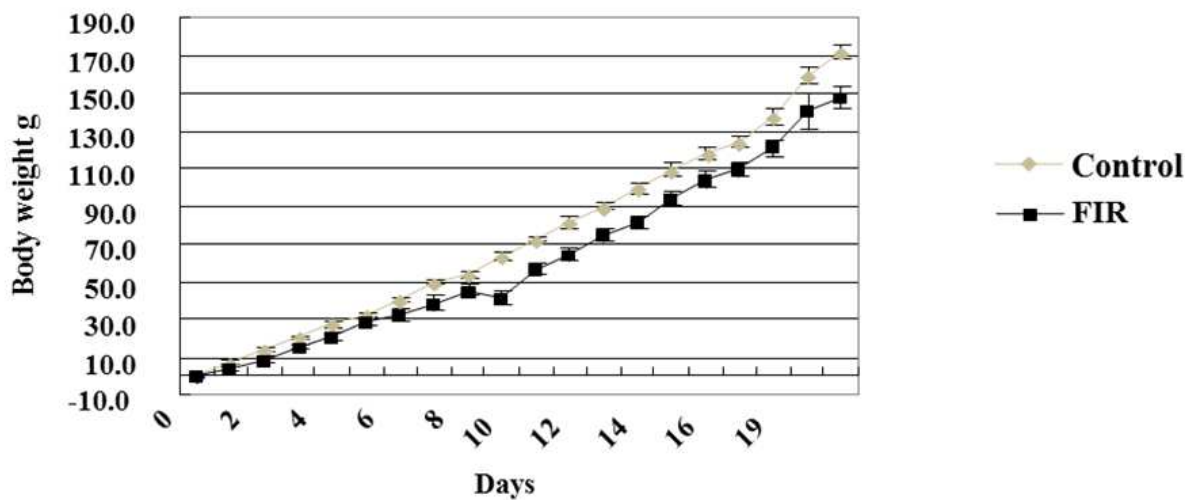
**Figure 12.** The solubility of BSA in the FIR energy radiated water

5. Experiment

5.1. The effects of the FIR energy radiation on an animal

5.1.1. The effect of the FIR energy radiation on the change of body weight of an animal

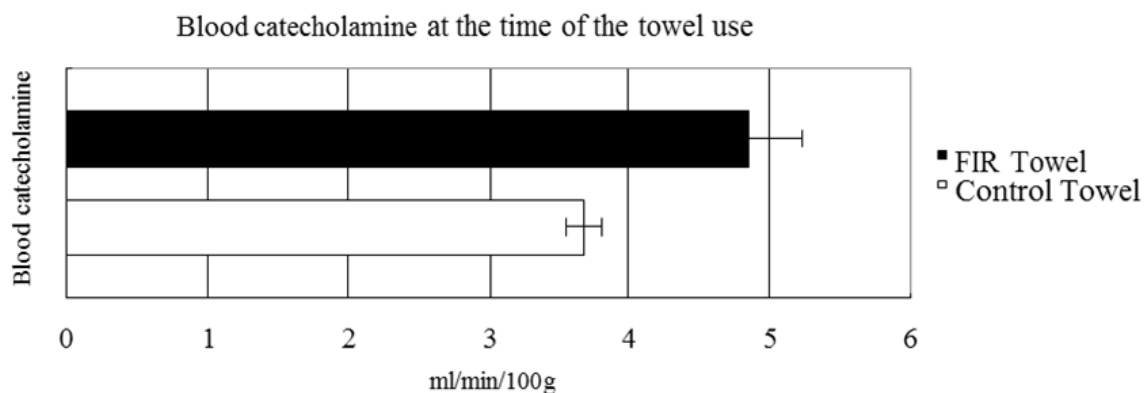
When the rats were raised by using the FIR animal raising equipment, the increase of the body weight of rats radiated FIR energy became gentler than that of control (Fig.13). Though the same experiments on the body weight of rats repeated five times, the results showed the same tendency that the FIR energy radiation inhibited the increase of the body weights. In some laboratories, the rats and the mice eat foods and drink water as much as wish. As a result, the rats and the mice naturally grow fat within a year. Therefore, it should be thought that the FIR energy radiation activates the exercise activity and keeps away the switchover to the obesity conditions.



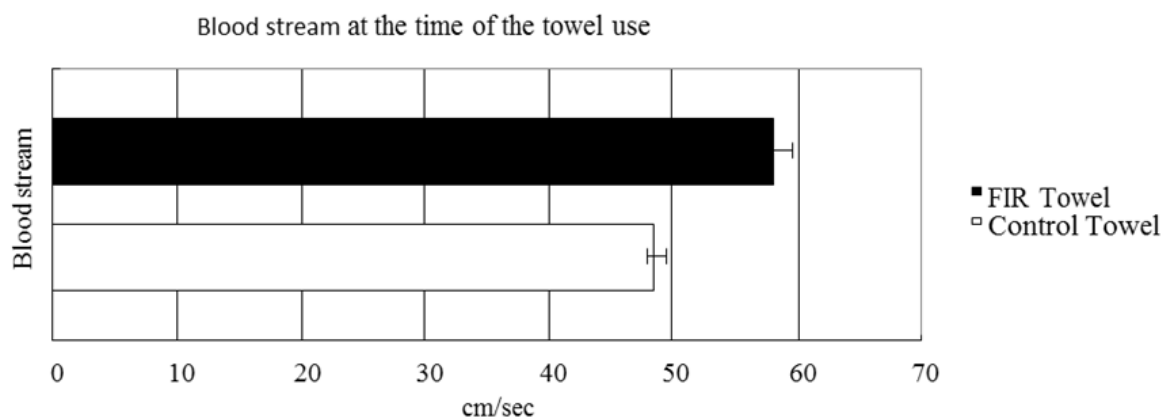
**Figure 13.** The change of the body weight affected by FIR. The growth of the rats was inhibited by the rhyolite as the FIR radiator.

### 5.1.2. The effect of the FIR energy radiation on the change of blood flow of an human

The Rhyolite powder with a peak diameter of about 10 $\mu$ m was printed to the blanket cloths made in Japan at 15%, like polka dots of about 2 cm (MATERA Inc. Toon, Ehime, Japan). The Rhyolite containing blanket and the control blanket were applied to the healthy volunteers for application of FIR energy of 20 min at room temperature 22°C and room humidity 50%, after acclimation of the condition in the room for 10 min. The velocity and the quantity of blood were continuously measured by the laser Doppler blood perfusion monitor and imagers in the same way for 20 min. The order of the application between the FIR and the control blankets was often changed at the condition of double blind. The velocity and the quantity of blood flow were accelerated 32.2% (Fig.14) and 19.1% (Fig.15) by the application of the Rhyolite containing cloths.



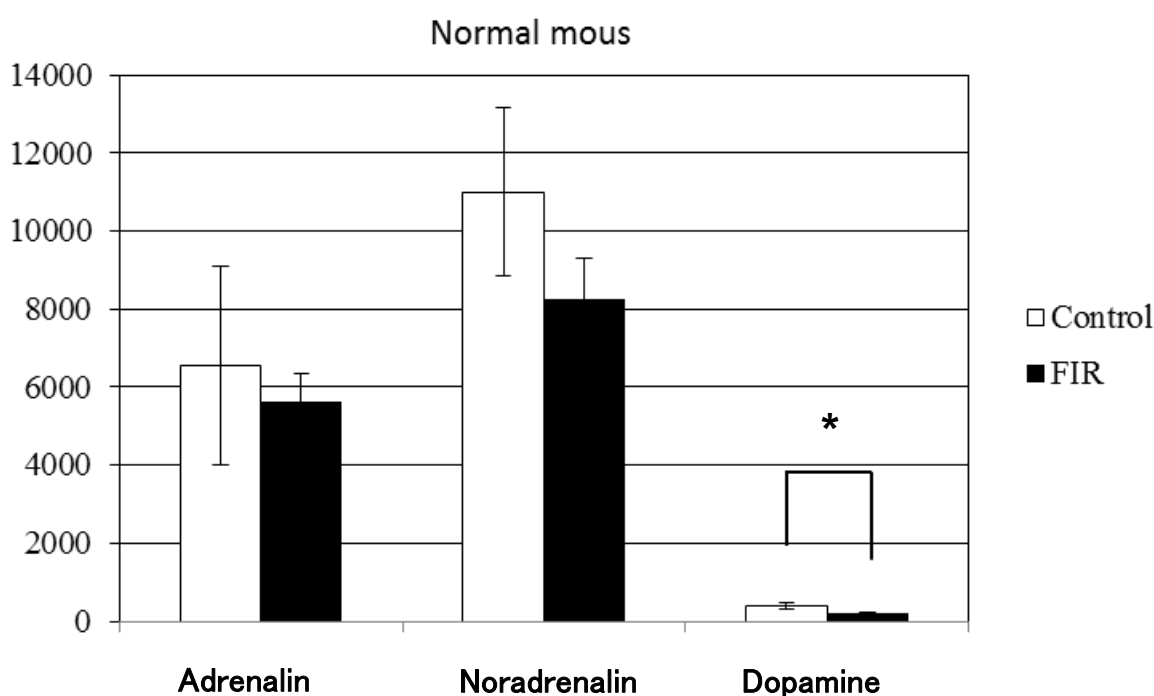
**Figure 14.** The change of the velocity of blood by the FIR blanket. This graph showed the velocity of the blood after 60 min of using the rhyolite containing blanket. The velocity of the blood increased 32.2% in the rhyolite containing blanket compared with the control blanket.



**Figure 15.** The change of the quantity of blood by the FIR blanket. This graph showed the quantity of the blood after 60 min of using the rhyolite containing blanket. The quantity of the blood increased 19.1% in the rhyolite containing blanket compared with the control blanket.

### 5.1.3. The effect of the FIR energy radiation on the change of blood catecholamine

The amount of adrenaline, noradrenaline and dopamine in blood of the 40 mice keeping in the FIR and control animal raising apparatus for 40 days were measured at the blood center. It was shown that the amount of the dopamine in blood catecholamine significantly decreased, and the adrenaline and the noradrenaline in the blood catecholamine tend to decrease by radiated the FIR energy (Fig.16). These results suggested that the FIR energy radiation decreased the amount of the blood catecholamine in mice to inhibit the sympathetic nerve and activate the parasympathetic nerve. A mamma as a human body has the autonomous nervous system with the motor nervous and sensory nervous system. The autonomous nervous system distributes all internal organs to work in order to sustain a person's life independent of his mind.



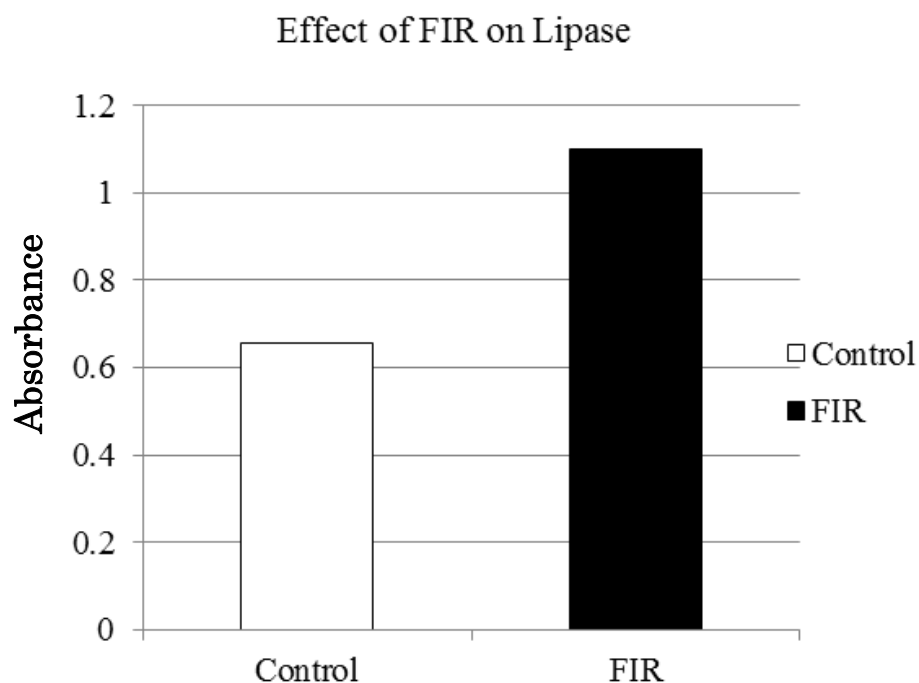
**Figure 16.** The change of the concentration of catecholamine in the blood affected by the FIR energy radiation. The concentration of the adrenaline, noradrenaline and dopamine in the FIR group were significantly lower 14.4%

There are the sympathetic nerves and the parasympathetic nerves in the autonomous nervous system which distribute every internal organ at the same times to control the function of each internal organ in spite of his mind. When the sympathetic nerves excited, the heart beat violently, the blood pressure increase, the breath become hard and the stress build up in the body. On the contrary, when the parasympathetic nerves excited, the feeling become comfortable as at home to become feeling an appetite, sleep and sexual desire. It is thought that the rats and the mice radiate FIR energy become loss of the stress in the cage and lives the active and healthy life. In short, FIR energy radiation may avoid the stress and obesity.



#### 5.1.4. The effect of the FIR energy radiation on the biological macromolecules

The effect of FIR energy radiation on the biological macromolecules is being made clear. The effects of FIR energy radiation on the activity of the enzyme can be estimated by using the FIR incubator developed by us. An enzyme is the special protein which has the various amino acids forming the three-dimensional structure with the various bonds to get a special role to a chemical reaction. In case that the activities of the 19 kinds of the enzymes appear in radiating FIR energy, it was made clear that the activity of some enzymes were accelerated or restrained. These results suggested that the efficiency of the enzyme activity changed because the various bond conforming the three-dimensional structure of the enzyme was affected by the FIR energy. In the enzymes measured by us, the results suggested that the activities of esterase, esterase lipase, lipase, leucin allylamidase, beta-glucuronidase were accelerated and that of alkaline-phosphatase, acid-phosphatase, naphthol-AS-BI-phospho hydrazase were restrained. Especially, esterase, esterase lipase and lipase are the enzymes to decompose a fat. Though the experiments were preliminary study, it was made clear that the FIR energy radiation changed the three-dimensional structure of the various enzymes to accelerate or restrain the activities. Still more, it was possible from the results that the increase of body weight restrained because some decomposition enzyme of fat were restrained by FIR energy radiation. These results suggested that the FIR energy radiation has a diet effect.

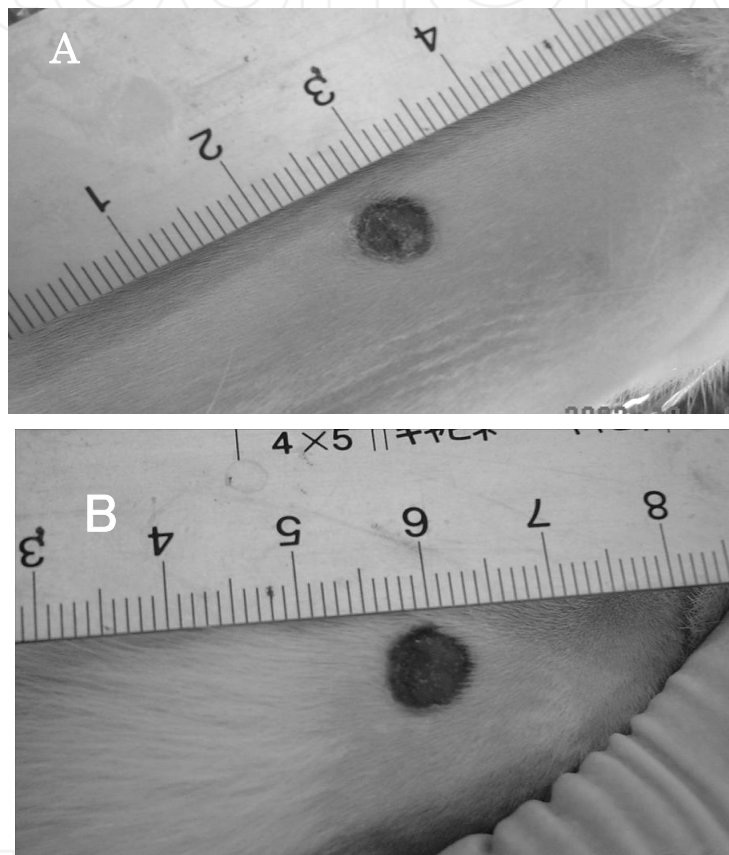


**Figure 17.** The change of activity of lipase by FIR energy radiation. The activity in the FIR radiation group was higher 40.5% than the control group at 530nm.

## 5.2. The effects of FIR energy radiation skin

### 5.2.1. The effect of FIR energy radiation on skin repair

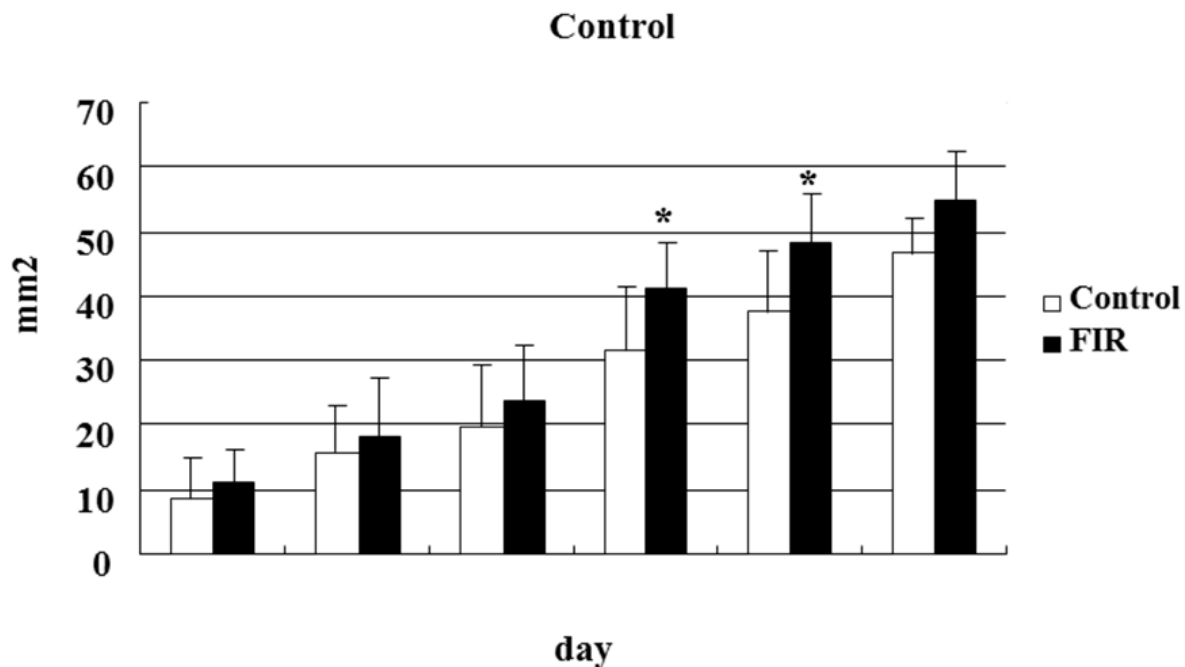
The wound of circle with 8mm diameter was artificially made at the skin of rats keeping the FIR and the control animal raising apparatus. Then, the area of the wound was measured each several days. And the surface condition was observed. The results showed that though the surface of the wound in the FIR radiation group wet and soft, that of the control group was covered the hard scab (Fig.18).



**Figure 18.** The surface of wound of skin affected by the FIR energy radiation. Control : (A) FIR energy radiation : (B)

The repair area of FIR radiation group increased each 30.54%, 28.27% and 14.22% after 5, 6 and 7 days compared with control group (Fig.19). These results showed that the FIR energy radiation from out of body accelerated the wound healing at the surface of rat skin. Still more, the repair area of wound increased each 44.39% and 24.29% after 3 and 5 days by application of the ointment containing the 10% rhyolite as the FIR energy radiating ceramics. In other words, it was clarified that the FIR energy radiation promoted the wound hearing of skin, even if the radiation of FIR energy were by far from body or by contact with skin. If the blanket and sheet with FIR energy radiation effects were developed, the bed sore of the bedridden old persons or the sick persons in bed for a long time would be suppressed and restored to regenerate the skin and ease the pain of the patients. Now, though the rise in

a fee for medical treatment becomes a social problem, if the bedsore could be suppressed to shorten the term of the admission to a hospital, the fee for the medical treatment would be reduced in the future. Still more, if the space of the residence was constructed with the FIR energy radiating building material as the rhyolite, the metabolism of skin would be promoted to hinder the aging of skin. Then, the FIR energy radiating ceramics as rhyolite may be tied together with a development of the creams and cosmetics to hinder the aging of skin.

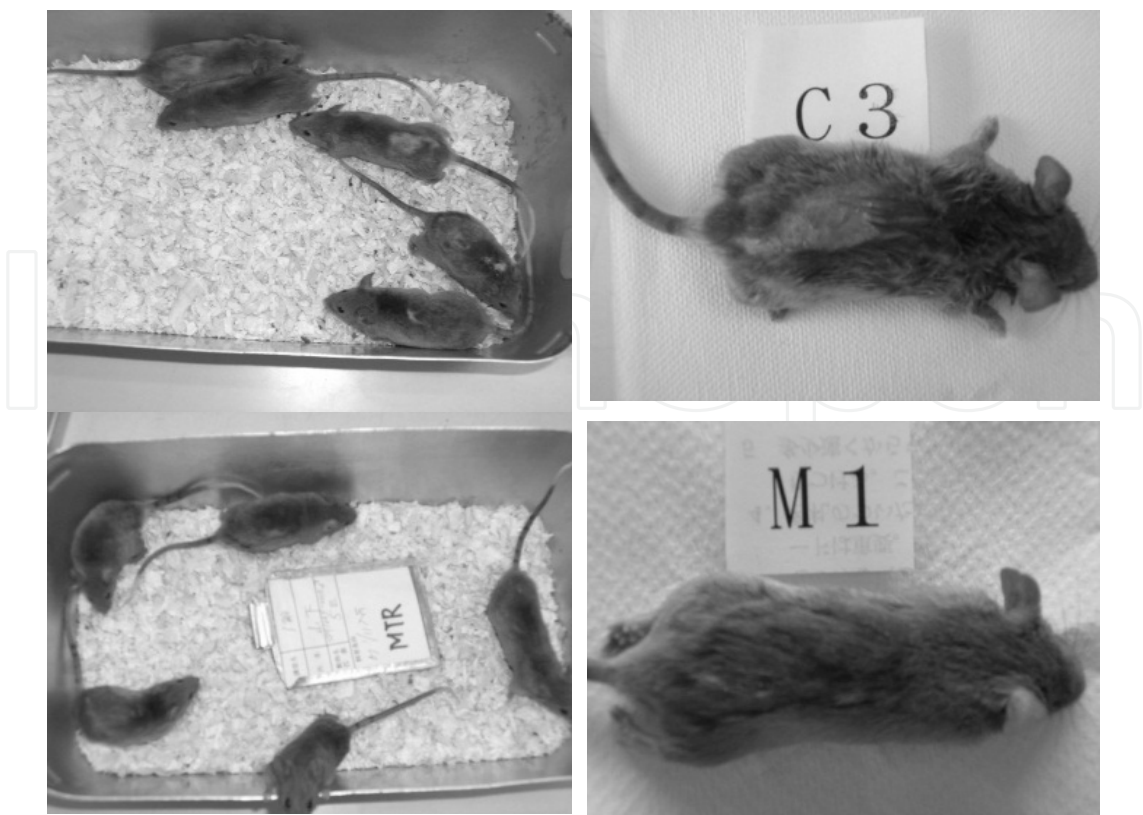


**Figure 19.** The repair area of wound healing. The speed of repair was accelerated affected by the FIR energy radiation.

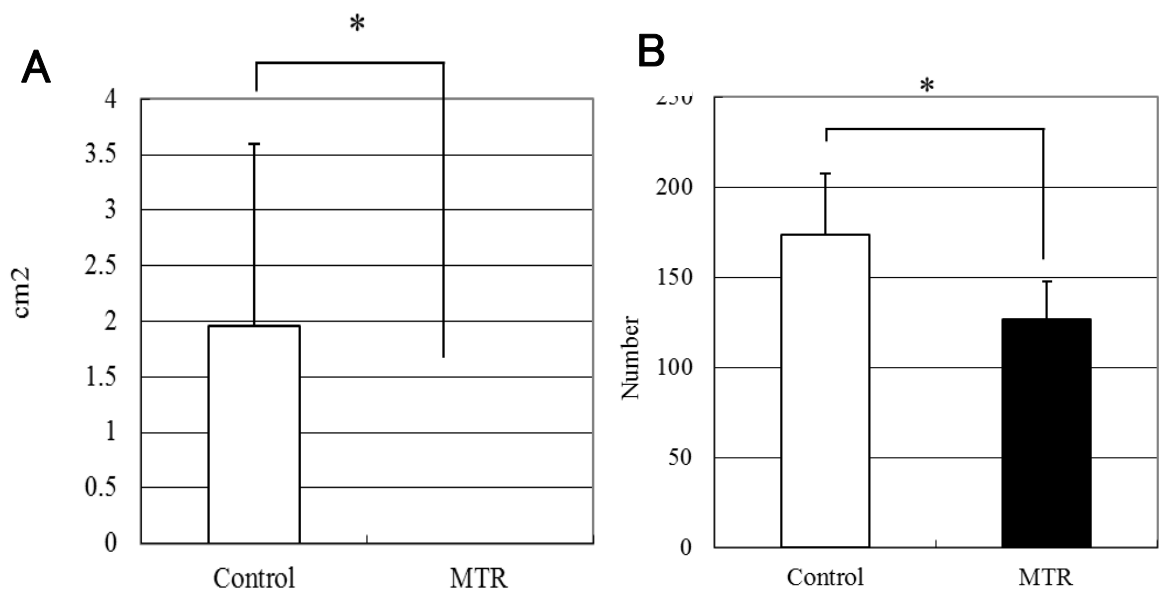
### 5.2.2. Effect of the FIR energy radiation on a atopic dermatitis

The mouse naturally induced a crisis of the inflammation of the skin as the atopic dermatitis with the application of a picric acid as the material raising the inflammation was imported from the United States. Then, the effects of the FIR energy radiation on the induction of the inflammation of the skin were analyzed by using the mouse. No an inflammation of skin in the mouse radiated FIR energy was detected after the application of a picric acid, though the atopic dermatitis of control mouse in the same condition were all induced without the FIR energy radiation (Fig.20). It was made clear that the area of the inflammation of skin and the number of the mast cells at the inflammation of skin as an atopic dermatitis clearly decreased by the FIR energy radiation (Fig.21).

These results suggested that the FIR energy radiation was effective for the prevention and the medical treatment to the inflammation of the skin as an atopic dermatitis. In the future, it is thought that the FIR ceramics as the rhyolite are tied together with the development of the medicine or the cure for the inflammation of the skin as an atopic dermatitis.



**Figure 20.** The change of the inflammation of the skin as the atopic dermatitis. The induction of the inflammation of the skin on all mice was suppressed by the FIR energy radiation



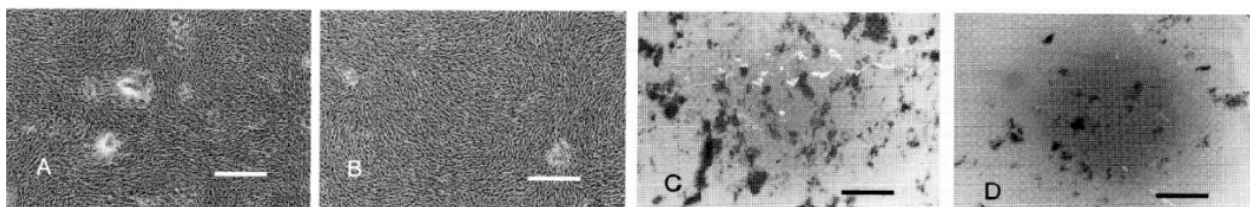
**Figure 21.** The area of the inflammation of skin and the number of the mast cells at the inflammation. Though the area of the inflammation in control group was 1.96cm<sup>2</sup>, no inflammation was detected in the FIR energy radiation group (A). The number of must cells was smaller 26.9% in the FIR energy radiation group than the control group (B).

### 5.3. The effects of the FIR energy radiation on bone

#### 5.3.1. The effect of the FIR energy radiation on the cultured osteoblast-like cells

The culturing osteoblast-like MC3T3-E1 cell (Riken Cell Banc, Tsukuba, Japan) were separated in the two groups in the FIR and the control CO<sub>2</sub> incubator. After 1, 3, 5, 7, 10 and 14days, the cell number of living cells in both groups were measured by a hemacytometer and observed by phase contrast microscope CK40 (Olympus, Tokyo, Japan). Still more, the gene expression of the 4 days culture cells in both groups was analyzed by the cluster analysis the microarray hybridization method (<http://www.godatabase.org>).

The proliferation of the osteoblast-like MC3T3-E1 cells in FIR group significantly inhibited each 19.08% and 12.24% after 3day and 10days culture compared with control group. But, it was considered that the formation of the calcified nodules in the FIR group was more than the control group by the observation of the culture cells stained with von kossa and with a phase-contrast microscope (Fig.22).



**Figure 22.** The effects of the FIR energy radiation on the bone nodules formed by MC3T3-E1 cells. The cultured MC3T3-E1 cells in FIR group (A) and control group (B) was observed by the phase contrast microscope CK40 (Olympus, Tokyo, Japan). Some samples after 4weeks culture were stained with Von Kossa method and observed by the same method. FIR group (C) Control group (D) Bar: 100 $\mu$ m

The results showed that the number and the area of the calcified nodules of the FIR group were 148.8 and 158.8  $\mu$ m<sup>2</sup>, though no calcified nodules was formed yet in control group after 2 weeks culture. Still more, that of FIR group increased each 2.92times and 4.91times compared with the control group after 4weeks (Fig.23). Consequently, the formation of the calcified nodules was clearly promoted by the FIR energy radiation. On the other hand, it was made clear by the results of cluster analysis after 4 day culture that the genes expression of Interferon activated gene 205, 203 and Interferon  $\alpha$ -inducible protein 27 in the cell to cell signal field were reinforced and the gene expression of Interleukin 1 family member 6, Interleukin 17D, 17 receptor C, 17B, and Interleukin 3 related with inflammation were oppositely dropped (Table 2).

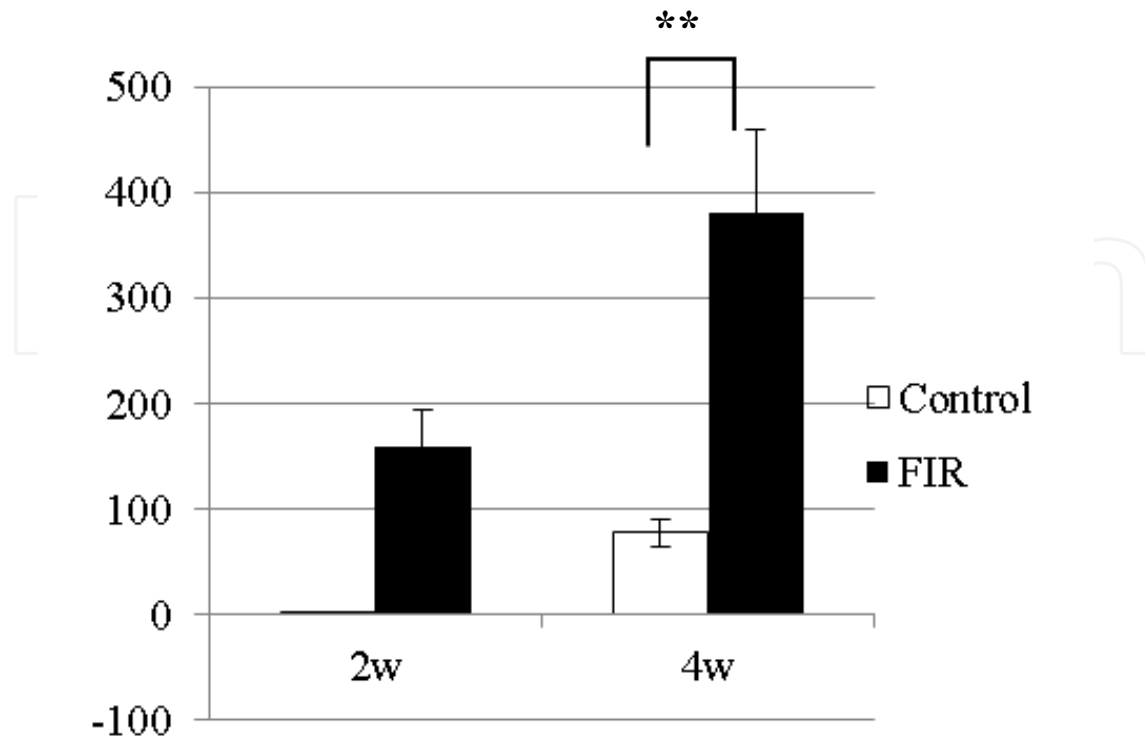
It was also made clear that the genes expression of Platelet-derived growth factor (PDGF) D polypeptide, Transforming growth factor  $\beta$  (TGF $\beta$ ) induced and TGF $\beta$  inducible early growth response 1 in the cell proliferation field were reinforced, and the gene expression of PDGF B polypeptide and Fibroblast growth factor (FGF) 2, 21, 8 were oppositely dropped (Table 2). It was clarified by these data that the cell differentiation as the function of osteoblast estimated by the formation of calcified nodules were promoted by FIR energy



radiation, though the proliferation was inhibited by it. In short, the FIR energy radiation shorts the term of cell proliferation and advances the timing of cell differentiation on the osteoblast. Finally, it was considered that the FIR energy radiation control these genes expression to inhibit the cell proliferation and promote the cell differentiation of osteoblast [8, 9]. It was made clear by these facts that the differentiation of cultured osteoblast-like cell as an osteoblast is promoted by the radiation of FIR energy.

<b>Cell-cell signaling</b> Gene Name	<b>Acc. No.</b>	<b>Fold change</b>
<b>Interferon activated gene 205</b>	NM_172648	1.597
<b>Interleukin 15 receptor, alpha chain</b>	NM_008358	1.422
<b>Interferon alpha-inducible protein 27</b>	AK010014	1.388
<b>Mitogen activated protein 3K 7</b>	BC006665	1.365
<b>Interferon activated gene 203</b>	NM_008328	1.328
<b>Interleukin 1 family, member 6</b>	NM_019450	-1.348
<b>Interleukin 17D</b>	NM_145837	-1.393
<b>Heparin-binding EGF-like growth factor</b>	NM_010415	-1.395
<b>Interleukin 3</b>	NM_010556	-1.471
<b>Interleukin 17 receptor</b>	NM_134159	-1.481
<b>Interleukin 17B</b>	NM_019508	-1.631
<b>Growth</b> Gene Name	Acc. No.	Fold change
<b>Platelet-derived growth factor, D polypeptide</b>	AK003359	1.555
<b>Transforming growth factor, beta induced</b>	NM_009369	1.505
<b>TGFb Inducible early growth response 1</b>	NM_013692	1.383
<b>Mitogen activated protein kinase kinase kinase 7</b>	BC006665	1.365
<b>Growth differentiation factor 15</b>	NM_011819	1.322
<b>Fibroblast growth factor 8</b>	D12483	-1.323
<b>Fibroblast growth factor 21</b>	NM_020013	-1.332
<b>Epidermal growth factor receptor related gene</b>	M99623	-1.337
<b>Fibroblast growth factor 2</b>	NM_008006	-1.342
<b>Heparin-binding EGF-like growth factor</b>	NM_010415	-1.395
<b>Early growth response 3</b>	NM_018781	-1.565
<b>Growth factor receptor binding protein2-ap</b>	NM_021356	-1.667
<b>1(Gab1)</b>	NM_011057	-2.304
<b>Platelet-derived growth factor, B polypeptide</b>		

**Table 2.** The cluster analysis of the gene expression in MC3T3-E1 cells affected by FIR energy radiation by the microarray hybridization method. These genes were reinforced and repressed at 1.3 over by the FIR energy radiation in the cluster of the Cell-to signaling and the growth.

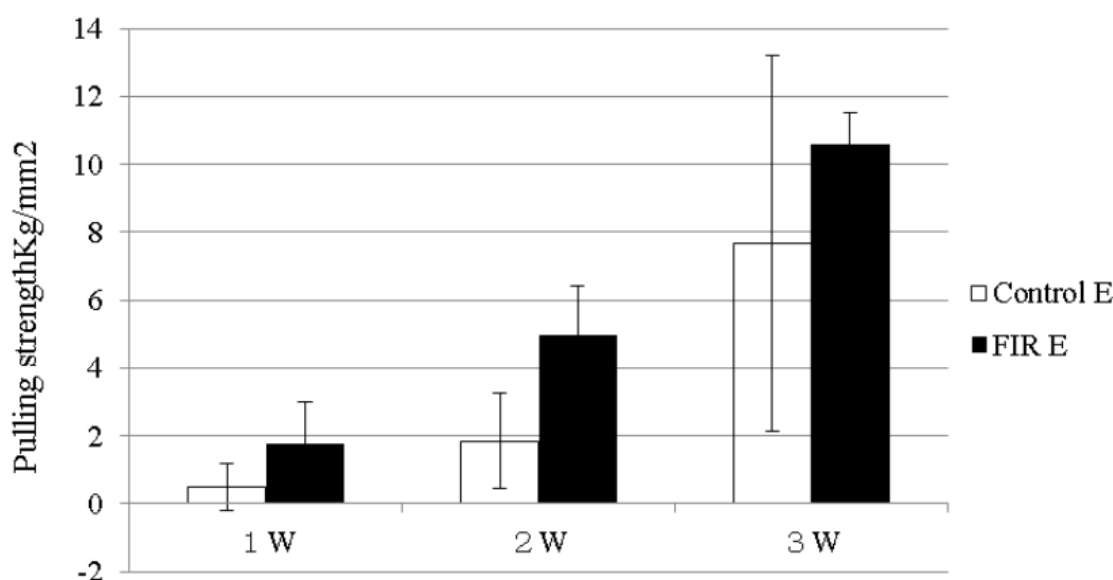


**Figure 23.** The effects of the FIR energy radiation on the number and the area of the bone nodules formed by MC3T3-E1 cells. Each five samples stained with Von Kossa method after 1, 2, 3 and 4 weeks in both groups were measured the nodules (A) and the number of the area (B) by Bio-system microscope BX-51. The measurement was analyzed by the average of five field of visions of each samples. Statistical analysis was carried out by t-test. \* $P < 0.01$

#### 5.3.1.1. The effects of FIR energy radiation on the titanium implant

Pure titanium post (RTP post titanium #Ti, Dentech, Tokyo) were treated with the snowtech O solution (Nissan Chemical Industries, Tokyo) for 48 hours and immersed in MEM medium (Minimum Essential Medium Eagle, Sigma, St. Louis, MO, USA) for 5 days. Control group was only immersed in MEM medium at same condition. The 20 S.D. rats of 6 weeks old were implanted the titanium post into the central position of the femur. Left side was control group and Right side was the snowtech O solution treated group. These rats were separated two groups which the FIR energy radiating group kept in the FIR animal raising apparatus and the control group was kept in the normal animal raising apparatus. The femur with the titanium post was extracted and measured the intensity between the titanium and the bone by the tension test with the small omnipotent testing machine (Autograph, Shimadzu, Kyoto) after 1, 2 and 4 weeks raising. The same experiments were repeated twice. Though the data were easy affected by the damage of the surgical operation and the extent of the inflammation, the identical tendency was shown. The tension intensity of the implanted titanium post with the snowtech O solution in the FIR energy radiating group significantly reinforced 2.68 times as much as the same implant in the control group after 2 weeks (Fig.24).





**Figure 24.** The effect of the FIR radiation on the strength of the bone bonding to titanium. The bone bonding to titanium was clearly promoted by the FIR energy radiation.

It was been thinking that FIR energy was too weak to permeate deep into the tissue of the living body. But, if the FIR energy radiation affects the water molecules in the blood as mentioned previously, the blood of the whole body was affected by FIR energy even if the deep position. The possibility is supported by the experimental results which the new bone formation inside of the femur was activated by the FIR energy radiation at the surface of the skin from outside of body.

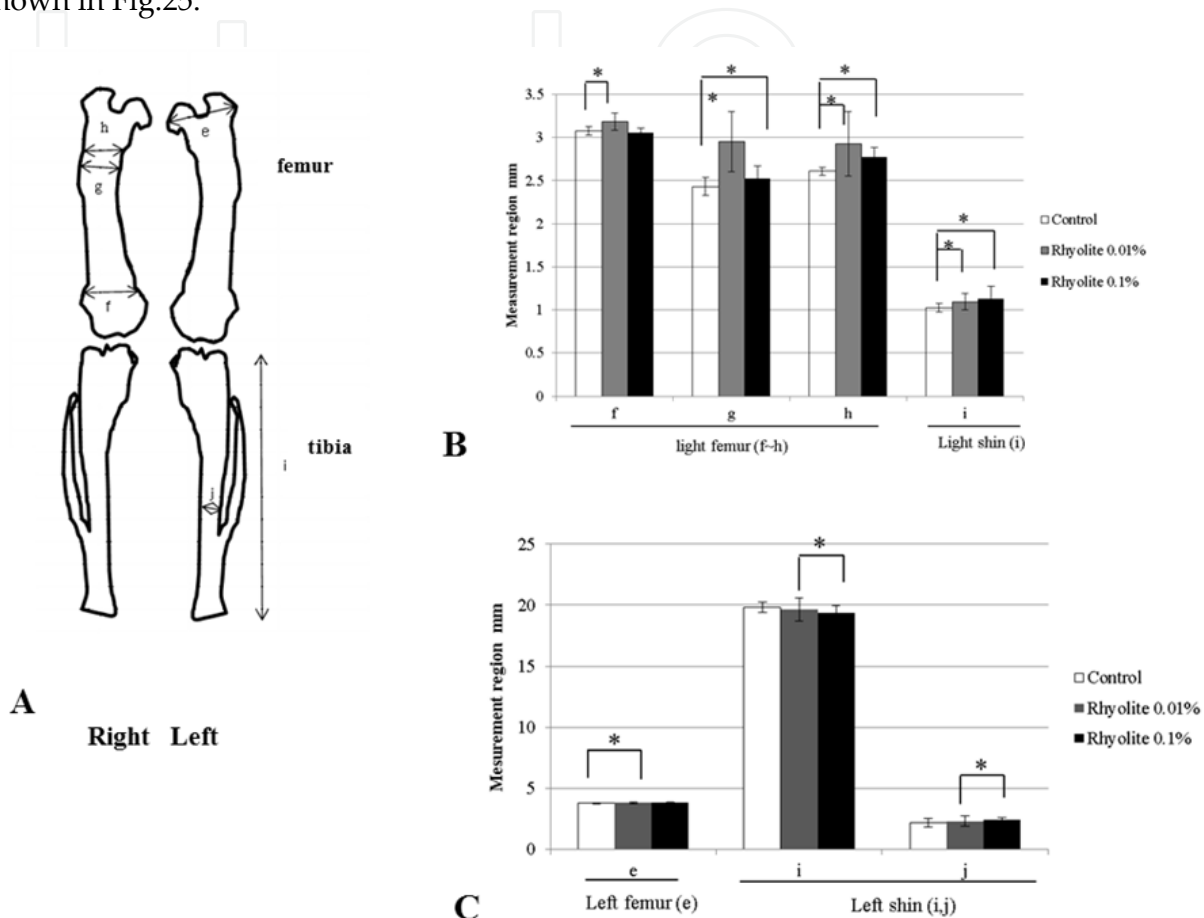
It is suggested that the deformational vibration of the water molecules in the blood affected by the FIR energy radiation was activated by the transmission of the energy through the resonance effect because the energy of deformation vibration was coincident with the radiating energy. It was proved that the blood containing the activated water molecules become quickly flow into the bone to activate the new bone formation.

#### 5.3.1.2. The effects of the feed containing the FIR ceramics on the growth of bone

The powder of the rhyolite mined at the vein of ore located on the Median Tectonic Line around Ehime prefecture was utilized for this experiment. The composition of the ingredient is SiO<sub>2</sub> 66.8%, Al<sub>2</sub>O<sub>3</sub> 13.52%, Fe<sub>2</sub>O<sub>3</sub> 6.98%, K<sub>2</sub>O 4.19%, Na<sub>2</sub>O 3.98%, CaO 2.55%. The 60 rats of SD strain at 4 weeks old were separated the three groups of 0.01% and 0.1%FIR treated and control. The each group was raised every 2 or 3 days with the standard feed containing 0.01% and 0.1% rhyolite and nothing for the each group in the measurement the amount of the eating feed. After 115days, all rats were killed by the over anesthesia and the femur and the tibia were measured at many parts of width and the length.

By the results of experiments, the amount of the eating feed of 0.01% FIR treated group increased up to 6.3%, that of 0.1% FIR treated group decreased on the contrary compared with the control group. The width of the right femur at distal, central and postal position significantly grew each 3.2%, 21.4% and 12.3% and that of the right tibia at central position

and the left femur at the distal position grew 1.3% also. The width of the right femur at central and distal position significantly grew each 3.7% and 6.1%, and that of the right tibia at central position and the left tibia at the distal position grew 11.8% also. Then, on the length of the long bone, the left tibia significantly grew 2.4% alone. The special affected parts were shown at Fig.25. It was clarified that the width of the femur and tibia quickly grew as shown in Fig.25.



**Figure 25.** The effect of the eating the feed containing rhyolite on the growth of the bone. The measurement parts of the femur and the tibia were shown (A). The growth of the femur and the tibia of these three groups of 0.01% and 0.1% FIR treated and control were compared after 115 days. Right : (B), Left : (C) It was clarified that the width of the femur and tibia quickly grew in the 0.01% FIR group.

It was made clear by these results that the bone formation was promoted by the inside radiation from the digestive organs by eating the feed containing the FIR energy radiating ceramics as same as the outside radiation of the FIR energy. These results suggest that the FIR energy radiation from the digestive organs arrives easy the deep posited bone of the living body. Still more, it was thought that the growth of length was a little inhibited and that of width was promoted. In other words, the FIR energy radiation does not promote the intrachondral ossification but the intra-membranous ossification that the osteoblast directly differentiates from the undifferentiated cells. As the effects of the bone affected more sensitive than the cartilage with the blood capillary, it was thought that the bone formation by the osteoblast is promoted by the FIR energy radiation. The results also prove that the

FIR energy radiation affected the living body though the blood containing many water molecules. It was clarified that the FIR energy radiation promotes the bone formation though the energy transmission to water molecules.

## 5.4. The Effects of the FIR energy radiation on the cancer

### 5.4.1. *The Effects of the FIR energy radiation on the culture cancer cells*

In order to clarify the effects of the FIR energy radiation on the culture cancer cells, A431 human epithelial vulva carcinoma cells, Sa3 human gingival squamous carcinoma cells, HSC3 human tongue squamous carcinoma cells, A549 human lung carcinoma cells, and MCF7 human breast carcinoma cells were cultured for the analysis of the effects on the proliferation, the shape of cells and the induction of apoptosis.

A431, A549, and MCF7 cells were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (Sigma, St. Louis, MO, USA). HSC3 and Sa3 cells were cultured in Eagle's basal medium (Sigma). All culture medium was supplemented with 10% heat-inactivated fetal bovine serum. The cells ( $5 \times 10^4$ ) were plated and measured on days 8 using 0.2% trypan blue and the hemocytometer. Incorporation of 5-bromo-2'-deoxyuridine (BrdU) was used to determine the amount of DNA synthesis. Cells were observed with a CK40 phase contrast microscope (Olympus, Tokyo, Japan), fixed, and stained with hematoxylin and eosin. Still more, apoptotic cells were identified using an Apo-BrdU in situ DNA Fragmentation Assay Kit (Bio Vision, Mountain View, CA, USA).

Although the proliferation of A549, HSC3, and Sa3 cells was significantly suppressed from day 6 of culture (59.0%, 75.4%, and 76.2%, respectively) up to at least day 10, FIR irradiation had little effect on the growth of A431 or MCF7 cells. Measurement of BrdU incorporation on day 4 of culture also showed a significant suppression of growth by FIR irradiation in A549, HSC3, and Sa3 cells but not in A431 or MCF7 cells. Observation of the morphology by the phase contrast microscopy revealed that the cytoplasm and nucleus was enlarged in A549 cells. Some of the HSC3 cells also showed hypertrophy of the cytoplasm and nucleus and others tended to show atrophy. Finally, some of the Sa3 cells showed hypertrophy of the cytoplasm (to refer JEMAA 2010, 2, 382-394, Fig.1) [10]

To determine whether the inhibition of proliferation by FIR energy radiation was associated with apoptosis or necrosis, cancer cells were subjected to FIR irradiation of 48 h and analyzed on day 4 by staining with annexin V-FITC and PI and by TUNEL (TdT-mediated dUTP-biotin nick end labeling) (to refer JEMAA, 2010, 2, 382-394, Fig.3)[10]. These results indicated that FIR did not induce apoptosis in A431, HSC3, or Sa3 cells. A few of the Sa3 cells showed significant necrosis. These results indicate that the inhibition of proliferation by FIR in the cancer cells, particularly in HSC3 and Sa3 cells, was not due to apoptosis. Still more, in order to analyze all expressing genes induced by FIR energy radiation, the control and the FIR-irradiated samples after four days culture was monitored using a Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA, USA) with elimination of low signal feature of background signal  $+ 2.6 \times SD$ , and P value  $< 0.01$ . Genes were further classified for process and function according to their

## A431

Genbank	Description	Fold change
NM_014316	Calcium regulated heat stable protein 1, 24kDa	1.777
AJ131186	PRP19/PSO4 pre-mRNA processing factor 19 homolog	1.754
NM_007056	Splicing factor, arginine/serine-rich 16	1.706
NM_003824	Fas (TNFRSF6)-associated via death domain	1.518
NM_130469	Jun dimerization protein 2	1.493
NM_031209	quacine (tRNA-ribosyltransferase 1 (tRNA-guanine transglycosylase)	1.491
NM_003564	transgelin 2	1.482
NM_021826	FAST kinase domains 5 (FASTKD5)	1.480
BT007321	general transcription factor IIH, polypeptide 4, 52kDa	1.383
AF099011	EH-domain containing 1	1.382
NM_153361	Hypothetical protein MGC42105	-4.525
NM_009589	arylsulfatase D	-4.016
NM_000125	estrogen receptor 1	-3.891
NM_032726	phospholipase C, delta 4	-3.745
NM_006477	RAS-related on chromosome 22	-3.472
NM_004797	Adiponectin, C1Q and collagen domain containing	-2.513
NM_015555	zinc finger protein 451	-2.506
NM_002285	AF4-FMR2 family member 3	-2.469
NM_012410	Seizure related 6 homolog (mouse)-like 2	-2.463
NM_005242	Coagulation factor II (thrombin) receptor-like 1	-2.066

## MCF7

Genbank	Description	Fold change
NM_006743	RNA binding motif (RNP1, RRM) protein 3	2.793
NM_012403	acidic (leucine-rich) nuclear phosphoprotein 32 family, member C	2.763
NM_012404	acidic (leucine-rich) nuclear phosphoprotein 32 family, member D	2.583
NM_014316	calcium regulated heat stable protein 1, 24kDa	2.304
BC022779	v-akt murine thymoma viral oncogene homolog 2	1.846
NM_001719	bone morphogenetic protein 7 (osteogenic protein 1)	1.817
NM_017590	zinc finger CCH-type containing 7B	1.736
NM_052960	retinol binding protein 7, cellular	1.598
NM_005724	tetraspanin 3	1.577
NM_032129	pleckstrin homology domain containing, family N member 1	1.479
NM_020317	chromosome 1 open reading frame 63	-1.938
NM_005803	fliotilin 1	-1.835
NM_001967	eukaryotic translation initiation factor 4A, isoform 2	-1.779
NM_005345	heat shock 70kDa protein 1A	-1.748
NM_015528	ring finger protein 167	-1.698
NM_032272	MAF1 homolog (S. cerevisiae)	-1.675
NM_024786	zinc finger, DHHC-type containing 11	-1.667
NM_005275	guanine nucleotide binding protein-like 1	-1.650
NM_003516	histone 2, H2aa	-1.645
NM_001964	early growth response 1	-1.623

## Sa3

Genbank	Description	Fold change
NM_006743	RNA binding motif (RNP1, RRM) protein 3	3.046
NM_014563	trafficking protein particle complex 2	1.964
NM_014316	calcium regulated heat stable protein 1, 24kDa	1.945
NM_001280	cold inducible RNA binding protein	1.825
NM_000475	nuclear receptor subfamily 0 group B, member 1	1.805
NM_145307	pleckstrin homology domain containing, family K member 1	1.788
NM_013962	neuregulin 1	1.731
NM_182687	protein kinase, membrane associated tyrosine/threonine 1	1.725
NM_024680	E2F transcription factor 8	1.710
NM_006123	iduronate 2-sulfatase (Hunter syndrome)	1.688
NM_000608	fibrinogen alpha chain	-3.571
NM_005039	proline-rich protein BstNI subfamily 1	-3.185
NM_002113	complement factor H-related 1	-2.899
NM_001885	crystalline, alpha B	-2.801
NM_001964	early growth response 1	-2.513
NM_004617	transmembrane 4 L six family member 4	-2.463
NM_006744	retinol binding protein 4, plasma	-2.410
NM_004543	nebulin	-2.353
NM_000204	I factor (complement)	-2.331
NM_021870	Fibrinogen gamma chain	-2.288

Genbank	Description	Fold change
NM_006121	keratin (epidermolytic hyperkeratosis)	2.383
NM_014316	calcium regulated heat stable protein 1, 24kDa	1.934
NM_001946	dual specificity phosphatase 6	1.805
NM_005547	involucrin	1.756
BC006322	activating transcription factor 3	1.753
NM_005555	keratin 6B	1.750
NM_003125	small proline-rich protein 11B (cornifin)	1.656
NM_014604	Tlx (human T-cell leukemia virus type 1) binding protein 3	1.605
NM_003315	DnaL (Hsp40) homolog, subfamily C, member 7	1.568
NM_002964	S100 calcium binding protein A8 (calgranulin A)	1.564
NM_005359	SNAD, mothers against DDP homolog 4 (Drosophila)	-1.976
NM_001348	death-associated protein kinase 3	-1.698
NM_130901	OTU domain containing 7	-1.695
NM_000524	5-hydroxytryptamine (serotonin) receptor 1A	-1.667
NM_006419	chemokine (C-X-C motif) ligand 13 (B-cell chemottractant)	-1.667
NM_032805	zinc finger protein 206	-1.623
NM_207370	G protein-coupled receptor 153	-1.618
NM_020999	neuregulin 3	-1.595
NM_005205	cytochrome c oxidase subunit VIa polypeptide 2	-1.592
NM_004118	forkhead-like 18 (Drosophila)	-1.590

Genbank	Description	Fold change
NM_014411	Nasopharyngeal carcinoma associated gene protein-8	4.253
NM_032972	protocadherin 11 Y-linked	4.037
NM_005854	receptor (calcitonin) activity modifying protein 2	3.662
NM_001485	gastrulation brain homeobox 2	3.570
NM_015831	acetylcholinesterase (YT blood group)	3.528
NM_032237	hypothetical protein FLJ23356	3.478
NM_024867	KPL2 protein	3.472
NM_004132	hyaluronan binding protein 2	3.345
NM_020602	phosphodiesterase 6G, cGMP-specific, rod, gamma	3.205
NM_178536	lipocalin 12	3.186
NM_002733	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	-2.309
NM_031266	heterogeneous nuclear ribonucleoprotein A/B	-2.212
NM_005556	keratin 7	-2.105
NM_006753	surfeit 6	-1.996
NM_006925	splicing factor, arginine/serine-rich 5	-1.988
NM_023044	stromal cell-derived factor 2-like 1	-1.984
NM_005694	COX17 homolog, cytochrome c oxidase assembly protein (yeast)	-1.828
NM_018645	Hairy and enhancer of split 6 (Drosophila)	-1.821
NM_005954	metallothionein 3 (growth inhibitory factor (neurotrophic))	-1.761

**Table 3.** The analysis of the extracted genes induced by the FIR energy radiation. The 10 most promoted genes and the 10 most inhibited genes in these changed genes showed

By the results that the significant changed genes of the 5 cancer cells by the FIR energy radiation were search, the cells inhibited the proliferation and showed that the many genes related the proliferation changed. The number of the inhibited genes by FIR energy radiation is more than that of the promoted gene except for the HSC3 cells. The 10 most promoted genes and the 10 most inhibited genes in these changed genes were shown at Table 3. There is no common gene in the expressed gene of 5 cells. These genes contained mainly the transcriptional factor and the control factor of the transcriptional factor.

#### *5.4.2. The control system in the cancer cells for the FIR energy radiation*

At the process of the analysis of the gene expression induced by the FIR energy radiation, it was made clear that the gene expression of HSP (Heat Shock Protein) 70 was reinforced by the FIR energy radiation. Therefore, the hyper expression cells and the knockdown cells of HSP70 were formed to analyze the effect on the proliferation.

In order to directly determine whether HSP70 can protect cells from FIR-induced cell death, we developed A431 and HSC3 cell lines stably expressing human HSP70A (A431-HSP70A and HSC3-HSP70A cells, respectively). Control cells were transfected with empty pcDNA3.1 (A431-Neo and HSC3-Neo). In our initial experiments, we found that the exposure of HSC3 and Sa3 cells but not A431 cells to the FIR energy radiation causes G2/M arrest and induces partial hypertrophy to the necrosis (data not shown) [12]. To determine whether the increased expression of HSP70A confers protection against the FIR energy radiation, the cell survival was examined in FIR-irradiated A431-HSP70, A431-Neo, A431-wt, and HSC3-HSP70, HSC3-Neo, and HSC3-wt cells. We found that over expression of HSP70A increased the cell proliferation in A431 and HSC3 cells. Furthermore, the proliferation of FIR-irradiated and control (unirradiated) A431-HSP70A cells was similar. The survival rate after 6 days of FIR irradiation was significantly higher in HSC3-HSP70A cells than in HSC3-Neo or HSC3-wt cells. In addition, the proliferation of FIR-treated HSC3-HSP70A cells was similar to that of control HSC3-HSP70A cells. The BrdU incorporation was significantly higher in FIR-irradiated or control A431-HSP70A cells than in A431-Neo or A431-wt cells. Although the BrdU incorporation of FIR-irradiated HSC3-wt and HSC3-Neo cells was lower than in unirradiated HSC3-wt and HSC3-Neo cells, it was similar in FIR-irradiated and unirradiated HSC3-HSP70A cells (to refer Med.Oncol 2008, 25, 229-237, Fig.3)[9].

HSP70 appears to be present in a variety of normal cell types and its expression may be induced by several stressors, such as hyperthermia, cardiac ischemia, infection, UV radiation, endotoxin, and nitric oxide to suppress or denature any foreign protein and restore an injured protein from lethal effects [13]. HSP70 seems particularly important for cancer cells. In human breast cancer, the expression of HSP70 correlates with increased cell proliferation, poor differentiation, lymph node metastases, and poor therapeutic outcome [14]. In vivo animal studies and clinical trials have revealed that hyperthermia may serve as a powerful tool in the treatment of prostate cancer [15-20]; at the cellular level, hyperthermic



stress induces HSPs. Moreover, chemotherapeutic agents such as cisplatin, adriamycin, and bleomycin, as well as  $\gamma$ -radiation induce HSPs. HSP70 participates in cytoprotection and is associated with cellular resistance to lethal external effects [18-21]. However, in the present study, HSP70 was never induced by FIR. These results suggested that FIR has anti-tumor activity without inducing HSP70 as an anti-stress factor. This characteristic indicates that FIR may be suitable for medical treatment.

We next examined the effect of knocking down HSP70A and HSP70C mRNA and HSP70 protein expression using siRNA. Transfection with HSP70A/C siRNA effectively decreased HSP70A and HSP70C mRNA (Fig. 4A) and protein levels in both A431 and HSC3 cells without affecting the level of HSP70B mRNA or protein. HSP70A/C siRNA did not suppress BrdU incorporation in unirradiated A431 cells, but it suppressed BrdU incorporation in cells irradiated with FIR. Similarly, the HSP70A/C siRNA enhanced the suppression of BrdU incorporation by FIR irradiation. FIR irradiation also significantly suppressed BrdU incorporation in HSC3 cells transfected with the negative control siRNA. These results indicate that a decrease in HSP70 protein mediates the ability of limited FIR to inhibit the proliferation of A431 and HSC3 cells (to refer Med.Oncol 2008, 25, 229-237, Fig.4)[9].

#### *5.4.3. The effects of the FIR energy radiation on the implanted cancer cell to mice*

For experiments on body weight change, 10-week-old male skid mice (CB17/Icr-Prkdc) and 6- to 8-week-old male nude mice (CrIj:CD1-Foxn1) purchased from Charles River Japan (Yokohama, Japan) were housed with temperature control and a 12-h light-dark cycle. Male skid mice were weighed every 5 days, beginning at 10 weeks of age. FIR treatment by our developed animal raiser (FIR group), n=7 and control (control group), n=8.

The log-phase cancer cells cultured in 10% FBS and DMEM/HamF-12 medium (Sigma, St. Louis, USA) resuspended at a cell density of  $1 \times 10^7$  ml in PBS containing 500  $\mu$ g/ml of Matrigel<sup>TM</sup> basement membrane matrix (Becton Dickinson Labware, Two Oak Park, Bedford, MA). The tumor cells ( $1 \times 10^6$ ) were suspended in 0.1 ml of PBS containing 50  $\mu$ g of Matrigel<sup>TM</sup> and were slowly hypodermically injected in the back using a 25-gauge needle. After injecting tumor cells, mice were separated two groups: FIR group and control group. During the experiments, the long and short diameters of the resulting tumor were measured in five-day intervals, and tumor volume was calculated by the following formula: tumor volume (mm<sup>3</sup>) =  $1/2 \times (\text{long diameter}) \times (\text{short diameter})^2$ .

For purpose of the ascertaining tumor organization change by the FIR energy radiation, the excised tumor was observed after stained with hematoxylin and eosin to detect tumor organization change after 30 days.

Then we carried out immunohistochemical analyses to detect matrix metalloproteinases (MMP) members MMP-1, MMP-9, MMP-10 and MMP-13 [9]. The increase in tumor volume

after implantation was significantly suppressed by whole-body FIR starting from day 15, compared to the control group (to refer J Table1). The cDNA microarray analysis of tumor samples of control and FIR-treated showed that MMP-1, MMP-9, MMP-10, and MMP-13 in the MMP family were significantly down-regulated in the FIR group compared with control group (to refer ITEL 2005, 6(6), 597-601, Table1) [9]. The expression profiles obtained from cDNA microarray analyses as analyzed by quantitative real-time RT-PCR showed the control group to have highly significant overexpression of the genes for MMP-1, MMP-9, MMP-10, and MMP-13 compared to the FIR group (to refer ITEL 2005, 6(6), 597-601, Fig.4) [9].

These results suggest that suppression of tumor volume increase by in vivo FIR radiation was due to inhibition of MMPs by FIR radiation. In other words, FIR suppresses invasion and metastasis of tumor cells by inhibiting the expression of MMP-1, 9, 10 and 13.

As shown in (to refer ITEL 2005, 6(6), 597-601, Fig 5) [9], extensive portions of tumor tissue in the FIR group was encapsulated and necrotized in intra tumor division. On the other hand, in the control group tumor cells showed active proliferation and invasion into the surrounding muscular tissue. In addition, evaluation of the immunohistochemical expression of MMP-1, MMP-9, MMP-10 and MMP-13 using tumor tissues of control and FIR groups on day 30 after implantation showed MMP-1 and MMP-9 to be positively expressed in the tumor stroma of the control group, while MMP-10 and MMP-13 were strongly positive in tumor parenchyma (the part positively dyed at Cytokeratin10) (to refer ITEL 2005, 6(6), 597-601, Fig. 6) [9]. However, these MMPs in the FIR group were not significantly expressed. The result of immunohistological detection for these MMPs was concordant with the results of cDNA microarray and QRT-PCR. That is, FIR radiation seemed to suppress invasion of tumor cells by inhibiting the expression of MMP-1, MMP-9, MMP-10, and MMP-13.

The increased expression and activity of MMPs is associated with tumor invasion, metastasis, and angiogenesis [22]. The role of MMP-1 in tumor invasion and metastasis has only recently been determined [23]. MMP-1 has been shown to cleave entactin, thus contributing to the degradation of basement membranes and hence potentially contributing to the transitioning across epithelial barriers by tumor cells [24]. Immunohistochemical detection of MMP-1 expression is also associated with increased invasive potential and poor prognosis in colon and esophageal cancers [25, 26]. The first barrier to tumor invasion is the basement membrane, and because one of its principle constituents is type I collagen, the gelatinases (MMP-2 and MMP-9) are thought to play important roles in its degradation [27]. MMP-10 (stromelysin-2) is known to degrade various components of the extracellular matrix, and though it has been reported that MMP-10 (stromelysin-2) expression by lymphoma cells accelerates the growth of thymic lymphoma [28], the role of MMP-10 in other types of tumor growth is relatively unknown. MMP-13 (collagenase-3) is expressed in breast carcinoma and in articular cartilage of arthritic patients [29]. In addition, MMP-13 has high collagenolytic and gelatinolytic activity, and MMP-13 may be of importance in



degradative processes involved in breast cancer progression [29]. In the present study, it was suggested that MMP-1, MMP-9, MMP-10, and MMP-13 are important for the invasion and metastasis of epithelial cancer of the human vulva A431 cell line in vivo and that the activities of MMP-1, MMP-9, MMP-10, and MMP-13 are selectively inhibited by FIR irradiation.

In conclusion, FIR irradiation can suppress tumor growth and invasion of epithelial cancer of the human vulva tumor by inhibiting the expression of MMP-1, MMP-9, MMP-10, and MMP-13 without critical side effects. Inhibition of MMP expression was considered to be one of the mechanisms by which multiplication of A431 tumor cells was suppressed in FIR irradiation.

## 6. Conclusion

It was made clear that the motion of the water molecules was surely and physically activated by the radiation of FIR with the CO<sub>2</sub> incubator and the animal raising apparatus. The analysis of the change of blood circulation conducted the conclusion that the FIR energy radiation activated not only the motion of water molecules but also the blood circulation in the living body. Still more, it was made clear that the activation effects on the water molecules developed the activation effects of the skin regeneration and the new bone formation. Moreover, the proliferation or metastasis of the various cancer cells were inhibited by the FIR energy radiation connected with the activation of blood circulation. It is expected that the present studies on the FIR energy radiation connect with the development of medical treatment for the regeneration of the tissue and organ as a skin and a bone, and the prevention medicine for cancer.

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